Effect of Cadmium on the Isolated Vas Deferens from Guinea-Pig

Atsuko NIWA, Takashi MIYAZATO, Noriko MURAKAMI*
and Aritomo SUZUKI*

Department of Parasitology, Department of Pharmacology,
Kinki University School of Medicine, Sayama, Osaka 589, Japan*

NIWA, A., MIYAZATO, T., MURAKAMI, N. and SUZUKI, A. *Effects of Cadmium on the Isolated Vas Deferens from Guinea Pig.* Japanese J. Smooth Muscle Res., 1981, 17, (2), 53-58
—— The effects of cadmium on the responses to the agonists, K, acetylcholine, noradrenaline, Ba and Ca were investigated in the isolated vas deferens from guinea pig.

1. The low concentration of cadmium enhanced the effects of tested agonists, and the enhancement was larger in order of K, Ba, Ca, noradrenaline=acetylcholine.

2. The high concentration of cadmium inhibited the effects of tested agonists with a dose-dependent manner. The inhibitory effect was weaker against the contraction by K and was stronger against the contractions by Ba, Ca, acetylcholine and noradrenaline.

3. The inhibitory effects of cadmium against the contractions by acetylcholine and noradrenaline were almost the same.

4. On the shape of contraction by K, cadmium showed the stronger inhibition against the tonic contraction than the phasic contraction.

5. The above mentioned results were mainly discussed on the Ca mobilization and the receptor selectivity. ——— Vas deferens, Cadmium, Ca, K, Acetylcholine, Noradrenaline, Ba

It is well known that cadmium is an extremely toxic element and the important role in the industrial process has meant that cadmium poisoning still remains as a serious problem.

It has been reported that growth retardation (Wilson *et al.*, 1941), renal damage (Axelsson & Piskator, 1966; Adams *et al.*, 1969; Castano, 1971), hypertension (Schroeder *et al.*, 1966, 1968a, 1968b) and infertility (Parizek, 1960) may be induced in human and animals when cadmium was administered by oral or parenteral route.

On the other hand, the effects of cadmium were investigated in the isolated smooth muscle organs. These authors reported that in a blood vessel (Perry *et al.*, 1963; Toda, 1973), a stomach (Shino, 1976) or an ileum (Schnieden & Small, 1971; Triggle *et al.*, 1975), cadmium might disorder the function of smooth muscle by inhibiting the Ca mobilization or by combining with the sympathetic alpha receptor. However, in spite of a cause of infertility little information is available concerning vas deferens.

In the present study, by using the isolated strips of vas deferens from guinea pig, the effects of cadmium on the responses to the some contractile agents were investigated, especially from the standpoint on the Ca mobilization and the selective inhibition against the receptor activity.

Submitted for publication, January 12, 1981.

丹羽淳子, 宮里 昂, 村上典子*, 鈴木有朋*

近畿大学医学部寄生虫学教室, 薬理学教室*

大阪府南河内郡茨山町西山 380

Material and Methods

Matured guinea pigs (weighing about 500 g) were sacrificed by a blow on the neck and vas deferens were removed.

The strip of vas deferens was fixed in an organ bath containing the nutrient solution (Locke solution). Isotonic contraction was recorded on the recorder with a magnification of ten times. The solution was maintained at 30°C and continuously bubbled with a mixture of 95% O₂ and 5% CO₂.

The Ca-free solution was prepared in the same fashion as Locke solution except that Ca Cl₂ was omitted.

The preparation was left alone in the bathing solution 1 hr at least before initiating the experiment.

The concentrations of drugs are expressed in terms of g/ml unless otherwise stated.

The drugs used were as follows: KCl (Wako), acetylcholine chloride (Sigma), noradrenaline hydrochloride (Sankyo), BaCl₂ (Wako), CdCl₂ (Wako), CaCl₂ (Wako).

Results

1. Effects of cadmium on the contraction by K.

The used concentration of K (2×10^{-3}) was approximately ED₈₀ of this agent.

The low concentration of cadmium (10^{-9} to 10^{-6}) enhanced the phasic and the tonic contraction by K. The high concentration (10^{-6} and more) inhibited them (Fig. 1 and Table 1).

The concentration for the maximal enhancement was about 10^{-7} and the heights of the

Fig. 1 Effect of cadmium on the contraction by K

Table 1. Effects of cadmium on the shape of contraction by K

Shape of contraction Concentrations of cadmium	Phasic contraction	Tonic contraction
10^{-9}	131.2 ± 10.5 (%)	124.6 ± 12.6 (%)
10^{-8}	144.0 ± 16.5	136.5 ± 15.1
10^{-7}	157.6 ± 18.4	163.6 ± 21.6
10^{-6}	133.0 ± 9.6	125.5 ± 11.7
10^{-5}	89.2 ± 4.5	81.5 ± 2.1
10^{-4}	73.3 ± 2.7	59.8 ± 3.4

The contraction height before the application of cadmium was calculated as 100%.

phasic and the tonic contraction were increased by 70%, respectively (Table 1).

The inhibitory effect of cadmium was increased with a dose-dependent manner, and 10^{-3} of cadmium completely inhibited the phasic and the tonic contraction. On the enhancement, the remarkable difference was not found between the phasic contraction and the tonic contraction. But, on the inhibition, cadmium showed the stronger inhibition against the tonic contraction than the phasic contraction (Table 1).

Fig. 2 Effect of cadmium on the contraction by acetylcholine

Table 2. Effects of cadmium on the responses to the some contractile agents

Agonists Concentrations of cadmium	Acetylcholine	Noradrenaline	Ba	Ca
10^{-8}	$104.8 \pm 2.2 (\%)$	$101.7 \pm 2.0 (\%)$	$122.0 \pm 7.2 (\%)$	$116.0 \pm 5.8 (\%)$
10^{-7}	103.4 ± 6.3	107.2 ± 2.2	124.3 ± 6.6	91.6 ± 6.3
10^{-6}	89.2 ± 6.3	85.4 ± 3.7	73.2 ± 2.8	71.9 ± 2.4
10^{-5}	28.5 ± 8.8	25.9 ± 2.4	30.4 ± 7.3	29.3 ± 7.9
10^{-4}	0	0	0	0

The contraction height before the application of cadmium was calculated as 100%.

2. Effects of cadmium on the contraction by acetylcholine.

The used concentration of acetylcholine (ACh) (10^{-7}) was approximately ED_{80} of this agent.

The low concentration of cadmium (10^{-8} to 10^{-7}) slightly enhanced the contraction by ACh or did not affect on it (Fig. 2 and Table 2). The high concentration of cadmium (10^{-6} and more) inhibited it, and 10^{-4} and more of cadmium completely inhibited it (Fig. 2 and Table 2).

3. Effects of cadmium on the contraction by noradrenaline.

The used concentration of noradrenaline (10^{-5}) was approximately ED_{80} of this agent.

The low concentration of cadmium (10^{-8} to 10^{-7}) slightly enhanced the contraction by

Fig. 3 Effect of cadmium on the contraction by noradrenaline

noradrenaline or showed little change. The high concentration (10^{-6} and more) inhibited it, and 10^{-4} and more of cadmium abolished it (Fig. 3 and Table 2).

4. Effects of cadmium on the contraction by Ba.

The used concentration of Ba (10^{-3}) was approximately ED_{80} of this agent.

The low concentration of cadmium (10^{-8} to 10^{-7}) enhanced the contraction by Ba. While the high concentration (10^{-6} and more) inhibited it. The concentration of 10^{-4} and more of cadmium abolished it (Fig. 4 and Table 2).

Fig. 4 Effect of cadmium on the contraction by Ba

5. Effects of cadmium on the contraction by Ca.

The used concentration of Ca (1 mM) was approximately ED_{80} of this agent.

In the normal medium, the tonus of the preparation was not affected by the application of Ca. But, with the preparation in which the contraction by K disappeared in the Ca-free medium, the exogenous Ca produced the contraction in the Ca-free, high K (5×10^{-3}) medium. Thus, the contraction by Ca was obtained by adding Ca 1 mM in the Ca-free, high K medium.

The low concentration of cadmium (10^{-8}) enhanced the contraction by Ca. But the high concentration (10^{-7} and more) inhibited it and the concentration of 10^{-4} of cadmium abolished it (Fig. 5 and Table 2).

Fig. 5 Effect of cadmium on the contraction by Ca

Discussion

The low concentration of cadmium enhanced the contractions by the agonists tested. But the high concentration inhibited them. As to these enhancing or inhibiting effects, the remarkable differences were found. That is, the enhancing effect was larger in order of K, Ba, Ca, ACh=noradrenaline, while the inhibiting effect was larger in order of ACh=noradrenaline, Ca, Ba, K. Also, there were some differences on the phasic contraction and the tonic contraction by K.

It is reported (Suzuki *et al.*, 1978) that the phasic and the tonic contraction by K are

maintained by Ca release and Ca influx. The fact that the low concentration of cadmium enhanced the phasic and the tonic contraction by K means that this concentration of cadmium accelerates Ca release and Ca influx. In addition, the result that the low concentration of cadmium enhanced the contraction by Ca supports to accelerate Ca influx of cadmium, because the Ca-induced contraction is maintained by only Ca influx. Niwa *et al.* (unpublished) reported that the low concentration of cadmium enhanced Ca release and Ca influx in the rat aorta. The minimal enhancing concentrations of cadmium to the contractions by K and Ca were 10^{-9} and 10^{-8} , respectively. The lower concentration of cadmium may accelerate more strongly Ca release than Ca influx.

In the concentration ranging from 10^{-7} to 10^{-6} , cadmium enhanced the phasic and the tonic contractions by K, while inhibited the contraction by Ca. This result suggests that in this concentration cadmium accelerates the Ca release but inhibits the Ca influx.

It is reported (Suzuki & Kinoshita, 1976) that the contractions by ACh, noradrenaline and Ba are due to only Ca release. Also, as to the division in the Ca store, which is utilized by the agonists, K, noradrenaline, ACh and Ba, the followings are mentioned: that is, K may release Ca for producing the contraction from the first store where Ca is most easily released, ACh and noradrenaline may release Ca from the first and the second store where Ca is relatively easily released, and Ba may release Ca from the first, the second and the third store where Ca is hardly released. Therefore the enhancing effects may suggest that the accelerating action of Ca release by cadmium is larger in order of the first, the third and the second store.

On the other hand, the weak acceleration of cadmium to the Ca release of ACh and noradrenaline may propose the other explanation. It is said (Perry *et al.*, 1963; Thind *et al.*, 1970; Suzuki *et al.*, 1978) that cadmium strongly combines with the sympathetic alpha receptor and the muscarinic receptor, resulting in the decreased excitation of receptors. Thus, in consequence of the decreased excitation, the accelerated Ca release by cadmium may be masked. There is a report (Suzuki *et al.*, 1978) that cadmium more strongly inhibited the contractions by ACh and noradrenaline compared with the contractions by K and Ba in the seminal vesicle from guinea pig.

The inhibitory effects of cadmium against the agonists were weaker to the contraction by K and were stronger to the contractions by ACh, noradrenaline and Ba. These results suggest that cadmium inhibits Ca release weakly from the first store and strongly it from the second and the third store. As stated above, the strong inhibition against the contractions by ACh and noradrenaline may be explained by the supposition that cadmium may strongly combine with the adrenaline and ACh receptors. Also, the strong inhibition against the contraction by Ba may show that cadmium compete with Ba on the cell membrane and reduces the Ba-induced Ca release, because cadmium is the same divalent ion as Ba.

The inhibitory effects of cadmium against the contractions by ACh and noradrenaline were almost the same. This fact suggests that the inhibitory effect of cadmium against the receptors is not selective. This is in agreement with the report (Suzuki *et al.*, 1978) in the seminal vesicle from guinea pig, but in disagreement with the reports by Thind *et al.* (1970) and Perry *et al.* (1963) that cadmium showed the selective inhibition against the sympathetic alpha receptor. However, these authors did not examine the inhibitory effect of cadmium

against the contraction by ACh, because rabbit aorta were used.

Suzuki *et al.* (1978) reported that the phasic contraction by K was due to Ca release in a large part and the tonic contraction was maintained by Ca influx in a large part. The high concentration of cadmium inhibited more strongly the tonic contraction than the phasic contraction. In other words, cadmium inhibits more strongly the Ca influx than the Ca release. The stronger inhibition against the contraction by Ca may support this thought. The agreement with ours has been reported in other observations in the seminal vesicle from guinea pig (Suzuki *et al.*, 1978), the rat stomach (Shino, 1976) and the rabbit aorta (Toda, 1973).

References

- Adams, R.G. *et al.* (1969). The development of cadmium-induced proteinuria, impaired renal function and osteomalacia in alkaline battery workers. *Q.J. Med.* **38**: 425–443.
- Axelsson, B. & Piskator, M. (1966). Renal damage after prolonged exposure to cadmium. An experimental study. *Arch. Environ. Health.* **12**: 360–373.
- Castano, P. (1971). Chronic intoxication by cadmium experimentally induced in rabbit. A study of kidney ultrastructure. *Path. Microbiol.* **37**: 280–301.
- Niwa *et al.* unpublished
- Parizek, J. (1960). Sterilization of the male by cadmium salts. *J. Reprod. Fertil.* **1**: 294–309.
- Perry, H.M. *et al.* (1963). In vitro production of inhibition of aortic vasoconstriction by mercuric, cadmium and other ions. *Proc. Soc. Exp. Biol.* **124**: 485–490.
- Schnieden, H. & Small, R.C. (1971). Spasmolytic effects of cadmium and zinc upon the guinea-pig isolated ileum preparation. *Brit. J. Pharmacol.* **36**: 488–499.
- Schroeder, H.A. *et al.* (1966). Hypertension in rats from injection of cadmium. *Arch. Environ. Health.* **13**: 788–789.
- Schroeder, H.A. *et al.* (1968a). Influence of cadmium on renal ischemic hypertension in rats. *Amer. J. Physiol.* **214**: 469–474.
- Schroeder, H.A. *et al.* (1968b). Action of a chelate of zinc on trace metals in hypertensive rats. *Amer. J. Physiol.* **214**: 796–800.
- Shino, H. (1976). Mechanisms of the contracting action of K, acetylcholine and Ba of the antispasmodic action of Cd and Mn on the pyloric antrum strip of rat's stomach, particularly in relation to Ca. *Folia Pharmacol. Japon.* **72**: 95–104.
- Thind, G.S. *et al.* (1970). Vascular reactivity and mechanical properties of normal and cadmium-hypertensive rabbits. *J. Lab. Clin. Med.* **76**: 560–568.
- Toda, N. (1973). Influences of cadmium ions on contractile response of isolated aortas to stimulatory agents. *Amer. J. Physiol.* **225**: 350–355.
- Triggle, C.R. *et al.* (1975). Intestinal smooth muscle contraction and the effects of cadmium and A23187. *J. Pharmacol. Exp. Ther.* **194**: 182–190.
- Suzuki, A. & Kinoshita, A. (1976). Mechanism of the contracting actions of K, acetylcholine, histamine, noradrenaline and Ba in the excised vas deferens of guinea pig, particularly in relation to Ca. *Acta medica Kinki Univ.* **1**: 43–53.
- Suzuki, A. *et al.* (1978). A possible mechanism of infertility by acute cadmium intoxication. *J. Toxic. Sci.* **3**: 313–324.
- Wilson, R.H. *et al.* (1941). Effects of continued cadmium feeding. *J. Pharmacol. Exp. Ther.* **71**: 222–235.